

FORM 2 – FULL RESEARCH PROPOSAL

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<p>Research title³ Effect of positive expiratory pressure on symptoms, extravascular lung water accumulation and cardiovascular function at high altitude</p>
<p>Lay summary⁴ High altitude exposure and hypoxemia can provoke pulmonary disorders responsible for acute mountain sickness and potentially high altitude pulmonary oedema. Breathing with a positive expiratory pressure as performed in critical care medicine has been shown to increase arterial oxygenation at high altitude but the underlying mechanisms and consequences on symptoms remain to elucidate. By using innovative methodologies (lung ultrasonography and speckle tracking echocardiography in particular) in subjects with or without acute mountain sickness, the present project aims to assess the effect of positive expiratory pressure on extravascular lung water accumulation and on the cardiovascular function at high altitude and to clarify its efficacy for managing altitude sickness.</p>
<p>Scientific proposal, background⁵ Positive expiratory pressure. Positive expiratory pressure (PEP) is used in critical care medicine to improve pulmonary gas exchange and compliance (9, 30). At least part of the effect of PEP on gas exchange is thought to result from an increase in alveolar pressure, leading to improved lung diffusion capacity and blood oxygenation (8, 28). PEP has also been proposed as a tool to improve oxygenation and symptoms in subjects with high-altitude pulmonary oedema (16, 36, 38).</p>

We recently shown in healthy subjects that 10-cmH₂O PEP (PEP10) in normobaric (FiO₂ = 0.12) and hypobaric (4350 m) hypoxia improves arterial (S_pO₂) and muscle (measured by near infrared spectroscopy, NIRS) oxygenation independently of changes in ventilation (23). It is well known that the severity of high altitude diseases (such as acute mountain sickness -AMS- or high altitude pulmonary oedema for instance) is closely correlated with the degree of arterial oxygen desaturation. Burtcher et al. (7) showed for instance that, for a given altitude (>2,500 m) or equivalent normobaric hypoxic level, a difference of about 4.9% of S_pO₂ is a key factor that distinguishes people who develop symptoms of altitude intolerance and those remaining clinically healthy. Therefore, one could suggest that increasing arterial and tissue oxygenation with PEP breathing at high altitude may improve symptoms and health status. This has been suggested in a single case study assessing the effect of pursed-lips breathing in a subject presenting severe AMS at 4,330 m (41). The subject reported a relief in symptoms and a rapid and critical increase in S_pO₂ (+23%) has been observed after 30 min of pursed-lips breathing but no other parameter was measured. Other case reports suggested that positive expiratory pressure may be useful to manage subjects with high altitude diseases (15, 16, 38). Savourey et al. (36) exposed 22 subjects for 8 hours to hypobaric hypoxia (4500 m) with or without a 5-cmH₂O PEP. They showed that PEP reduced the severity of AMS severity reported on the Lake Louise questionnaire. Additional studies are therefore needed to assess the effect of PEP breathing on symptoms with large sample size and under real high altitude conditions to confirm its usefulness as a tool for acclimatization and management of high altitude diseases. In our recent study (23), we also measured significant increase in oesophageal pressure with PEP10 suggesting that PEP may influence gas exchange and arterial oxygenation by enhancing alveolar pressure. An increase in alveolar pressure might resorb some of the extra-vascular fluid accumulation and consequently improve oxygen diffusion and symptoms at high altitude (1). This effect of PEP on extra-vascular fluid accumulation and its consequence on oxygenation and symptoms at high altitude remains however to demonstrate.

Extravascular fluid accumulation at high altitude and thoracic ultrasonography.

Initially devoted to diagnose alveolar-interstitial syndrome (17) in pathological situations such as acute respiratory distress syndrome or cardiogenic acute pulmonary oedema (12), thoracic ultrasonography has been used to show extravascular lung water accumulation at high altitude (10, 26). The main criterion in favour of pulmonary alveolar-interstitial oedema was the presence of ultrasound lung comet (USLC) (27) which is particularly robust for assessing changes in alveolar-interstitial fluid (2, 14).

Pulmonary extravascular fluid accumulation in high altitude pulmonary oedema

depends on the quantity of liquid escaping from the pulmonary vasculature and on the ability of the alveolar respiratory epithelium to reabsorb flood overload (37). It is well established that hypobaric hypoxia increases pulmonary capillary hydrostatic pressure (20) and causes endothelial dysfunction promoting pulmonary vascular leakage (31, 40). In 18 healthy recreational climbers participating to a 3-week trekking up to 5130 m, the numbers of USLC at rest progressively increased with altitude ascent, even in 14 asymptomatic subjects, with no correlation with pulmonary artery systolic pressure increase (29). We recently reported a follow-up evaluation of asymptomatic alveolar-interstitial pulmonary oedema using ultrasonography during a 4-day period at a constant high-altitude (4350 m), without any medication or exercise-induced fatigue (6). In this context, we reported a peak USLC score on the first day at 4350 m followed by a progressive decrease until day 3 in 11 healthy subjects. The amount of USLC correlated with AMS severity (Lake Louise score) and SpO₂ but not with changes in left ventricular (LV) diastolic function or pulmonary arterial pressure. These results indicate that extravascular pulmonary fluid accumulation is highly prevalent in fast-ascending high-altitude recreational climbers and resolved within few days in asymptomatic subjects. Since no subject developed severe AMS in our study, the link between USLC and AMS remains to demonstrate. It has been suggested that PEP may improve gas exchange and symptoms at high altitude by resorbing extravascular lung water accumulation (1). Hence, thoracic ultrasonography offers the opportunity to assess the effect of PEP on extravascular lung water accumulation at high altitude in subjects with and without AMS symptoms and therefore to clarify the mechanisms of action of PEP in hypoxia as well as the link between USLC and the development of AMS.

Cardiac function at altitude and speckle tracking echocardiography.

PEP, especially when used in critical care, is known to have potential hemodynamic effect with impairment of cardiac function related to lung volume and intrathoracic pressure (ITP) changes (18). Briefly, when looking at the right side of the heart, the PEP can induce a reduction of the venous return due to an increase in right atrial pressure secondary to increased ITP. Also, high levels of PEP may result in pulmonary overdistension with an increase in pulmonary vascular resistance (and therefore of ventricular afterload) and left shift of the interventricular septum. When looking at the left side of the heart, the PEP can cause a reduction of stroke volume secondary to the shift of the interventricular septum and the increased pericardial pressure generated by the augmented ITP. In addition, PEP can reduce left ventricle (LV) afterload by increasing the pressure gradient between LV and aorta. In our recent study, we evaluated the effect of PEP on global cardiac function by using bioimpedance evaluation and we observed no difference between PEPs from 0 to 10 cmH₂O, although all PEP conditions caused a slight reduction in cardiac output

compared to free breathing (23). Further studies with more sensitive methods are needed to assess the effect of PEP breathing especially during hypoxic exposure on the cardiac function.

The initial cardiovascular response to altitude is characterized by an increase in cardiac output with tachycardia and no change in stroke volume. After a few days of acclimatization, cardiac output returns to normal, but heart rate remains increased leading to decreased stroke volume (22). If systolic LV function seems to be maintained or only slightly depressed until Everest summit (5), LV relaxation is impaired and reduces early diastolic filling (5, 6, 13). There is also evidence for altered right ventricular (RV) diastolic function due to increased pulmonary artery pressure (4). Nevertheless, since myocardial functionality results from a complex interplay between deformation (longitudinal and circumferential) and twist/untwist mechanics (3), these studies only partially described the effects of hypoxia on myocardial function. Indeed, an elegant study reported increased LV deformation during acute hypoxic breathing in normal subjects. Moreover, recent works demonstrated that sub-endocardial fibers have greater sensitivity to hypoperfusion (39), probably existing in hypoxic state, suggesting the relevance to evaluate mechanics in myocardial fibre layers.

Speckle tracking echocardiography (STE) is a new non-invasive ultrasound imaging technique that allows for an objective and quantitative evaluation of myocardial function, less dependent of the angle of insonation and of cardiac translational movements, compared to Doppler approaches. In this context, STE may help to evaluate the underlying mechanisms explaining LV and RV dysfunction in response to hypoxic exposure and to PEP breathing, separately and when combined, as STE offers a comprehensive and sensitive evaluation of regional myocardial function at both sub-endocardial and sub-epicardial levels (45).

Hypoxia, brain and PEP.

The brain is probably the most oxygen sensitive organ and several recent data suggest that it may be a key actor regarding altitude acclimatization and maladaptation (42, 44). We recently described changes in cerebral oxygenation by using NIRS during hypoxic exposure, showing the large sensitivity of the brain to changes in arterial oxygenation compared to the muscles for instance (34). Some data suggest that PEP breathing may affect cerebral perfusion and potentially oxygenation under hypoxic conditions (21, 24). Therefore, a specific evaluation of the cerebral impact of PEP breathing on cerebral perfusion and oxygenation at high altitude is needed to confirm both the efficiency and the safety of this method in subjects at high altitude (19).

Scientific proposal, aims and hypotheses⁶

This study aims to evaluate the effect of PEP breathing as a potential non-pharmacological method to improve oxygenation and symptoms at high altitude and

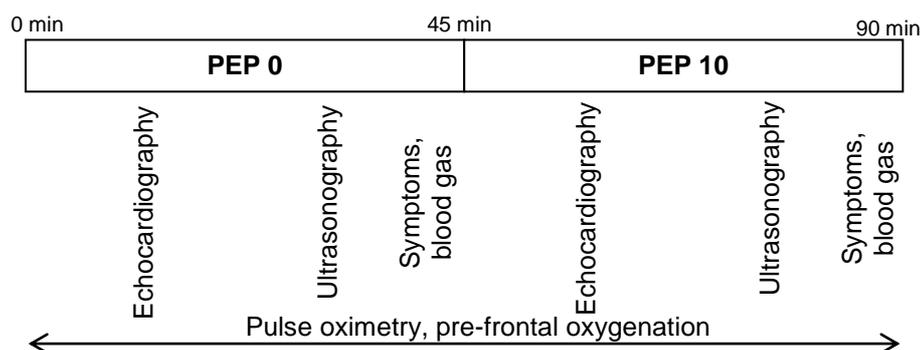
to describe the mechanisms underlying these effects of PEP. This work will focus specifically on the influences of PEP on symptoms of AMS, extravascular pulmonary fluid accumulation, cardiac function and cerebral perfusion and oxygenation. Subjects with various degrees of AMS at high altitude will be tested in order to assess the effect of PEP depending on the presence of symptoms and altitude sickness. The following hypothesis will be tested:

- PEP would improve arterial and cerebral oxygenation in all subjects at high altitude,
- PEP would resorb part of the extravascular fluid accumulation as observed by ultrasonography,
- PEP would improve symptoms in subjects with high-altitude sickness,
- High altitude exposure would provoke alteration in biventricular myocardial deformation in sub-endocardial and sub-epicardial layers,
- PEP (at 10 cmH₂O) would have no additional effect on cardiac function at high altitude.

Scientific proposal, methods⁷

Subjects. 36 subjects will be investigated at sea level and at high altitude (at Manaslu base camp -5000 m-, after a 9-day trek to this altitude), with various degrees of AMS. Subjects will be pre-screened at base camp by using the Lake Louise Questionnaire. The objective will be to investigate 12 subjects with Lake Louise score >3, 12 subjects with Lake Louise score between 1 and 3 and 12 subjects with Lake Louise score <1. Based on previous experience of the MEDEX group in similar conditions (25), the 3 groups with the requested Lake Louise scores should be easily obtained with the total amount of volunteers planned during the MEDEX MANASLU 2015 experiment (>50 subjects).

Study design. During the experimental protocol, subjects will lay supine and will breathe with a mouthpiece through a three-way valve connected on the expiratory side to a mechanical resistance inducing a PEP of 0 (placebo) or 10 cmH₂O. Subjects will breathe for 45 min with PEP0 and then for 45 min with PEP10 in order to perform all measurements in each condition (see graph below). Because of potential carryover effect, the order of the two conditions will not be randomized. Subjects will be kept blinded regarding the PEP level. Arterial oxygenation (pulse oxymetry) and cerebral oxygenation (pre-frontal NIRS) will be assessed continuously during PEP0 and PEP10. Blood gas analysis, arterial pressure and symptoms (Lake Louise score, visual analogue scales for headache and for dizziness) will be assessed at the end of the PEP0 and PEP10 phases. Echocardiography assessments will be performed after 10 min of PEP0 and 10 min of PEP10 breathing. The presence and amount of ultrasound lung comet will be assessed by thoracic ultrasonography during the last 10 min of the PEP0 and PEP10 phases.



Echocardiography. Left and right ventricular evaluations including standard echocardiography (Vivid Q, GE Healthcare, Horten, Norway), tissue Doppler imaging and 2D-strain echocardiography on sub-epicardial and sub-endocardial layers will be performed as previously described by a fully trained operator. The inferior vena cava diameter and the percent collapse of the inferior vena cava with inspiration will also be measured by ultrasound.

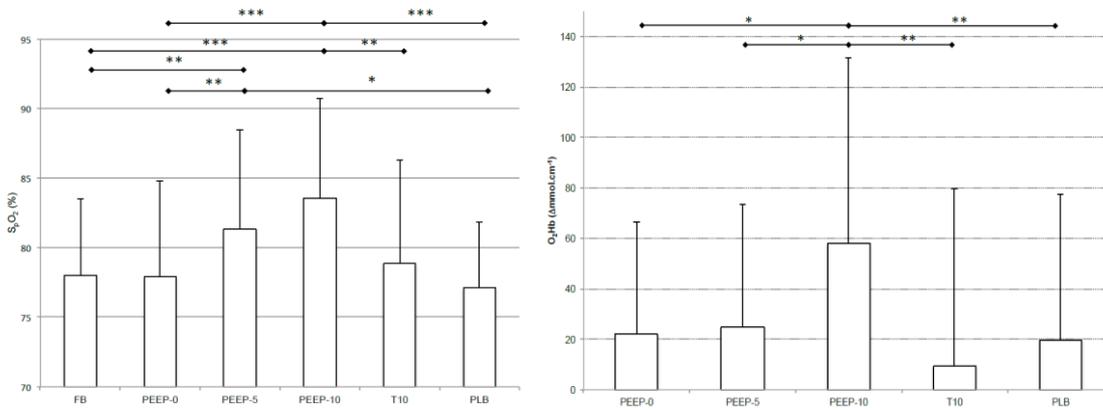
Thoracic ultrasonography. The upper, medium and lower parts of the anterior and lateral regions of the two chest walls will be sequentially examined (CX-50, Phillips, Eindhoven, Netherlands) by a fully trained operator with the subject in the supine position. USLC is defined as an echogenic, coherent, wedge-shaped signal with a narrow origin from the hyperechoic pleural line (27). The comet-tail should extend to the edge of the screen (whereas short comet-tail artefacts may exist in other regions) and arise only from the pleural line. This ultrasound sign correlates with alveolar-interstitial oedema assessed by chest radiography, wedge pressure and extravascular lung water measured by thermodilution (2). The number of USLC will be recorded through the sequential examination of 28 intercostal lung fields located at the parasternal, midclavicular, anterior axillary and midaxillary lines from the second to the fourth intercostal space on the left side and from the second to the fifth intercostal space on the right side.

Near infrared spectroscopy. A portable NIRS device (PortaLite, ARTINIS, The Netherlands) will be used to monitor relative concentrations in pre-frontal oxygenated-haemoglobin (ΔO_2Hb), deoxygenated-Hb (ΔHHb) and total-Hb ($tHb = O_2Hb + HHb$). Theoretical and performance details of NIRS have been previously described (32). NIR-determined hemodynamic reflects the dynamic balance between O_2 demand and O_2 supply in the tissue microcirculation and can be used at high altitude to assess cerebral oxygenation and perfusion changes (33).

Blood oxygenation. Continuous pulse oximetry will be performed by using a standard monitor (Mindray, China) while arterialized blood samples taken at the earlobe will be analysed for O_2 and CO_2 partial pressures as well as pH using a standard system (Radiometer, Copenhagen).

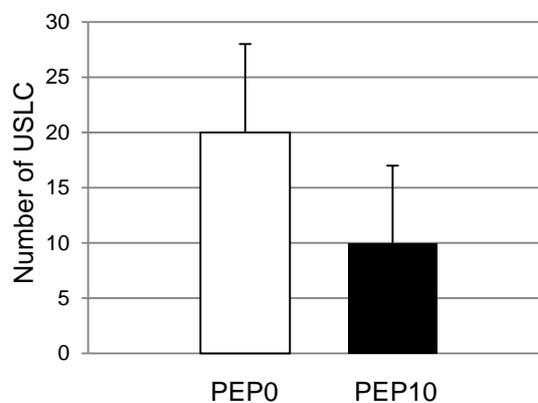
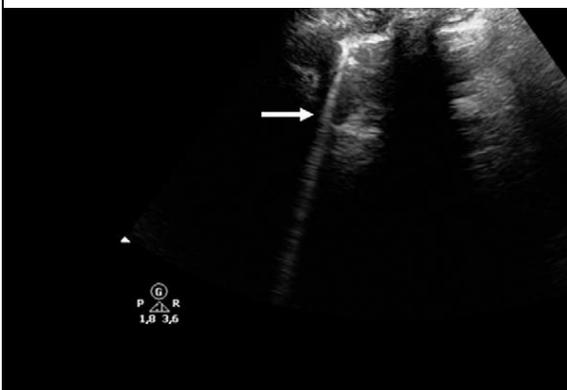
Scientific proposal, expected results^B

PEP is expected to increase arterial blood oxygenation as well as cerebral oxygenation similar to the results we obtained below during PEP 10 breathing in acute normobaric hypoxia for arterial oxygen saturation and muscle NIRS oxygenation



Arterial oxygen saturation (left panel) and quadriceps oxyhemoglobin (right panel) changes during PEP 0-10 cmH₂O breathing, free breathing (FB), pursed lip breathing (PLB) or similar ventilation than PEP10 but without PEP (T10) (From (23)).

Ultrasonography should allow detecting USLC as shown on the figure below. Based on our experience of ultrasonography at high altitude and previous other studies from the literature, we expected an average number of USLC of about 20 that might be reduced by about 50% after PEP10 breathing



Left panel: Typical ultrasound image of lung-comet tail (USLC, white arrow) in a subject at high altitude. Right panel: Expected number of USLC measure at 5000 m during sham or effective PEP.

Echocardiography should allow us the detection of classical myocardial dysfunction with alteration levels depending on AMS score. Based on previous works, PEP10 score may limit these myocardial adaptations, at least in subjects with higher AMS scores. Moreover, off-line analysis could show limitation in heart longitudinal deformation, especially in sub-endocardial layer associated with higher circumferential deformation and twist/untwist changes.

The overall results of the present project should establish whether PEP could be an important non-pharmacological tool to improve oxygenation and symptoms in subjects with altitude sickness. The safety of this method should be confirmed and the mechanisms of action should also be demonstrated in order to set PEP breathing as a potential useful intervention in addition to pharmacological treatments for the management of altitude sickness.

Dissemination plan, target journal(s)⁹

Journal of Applied Physiology, International Journal of Cardiology, High Altitude Medicine and Biology

Dissemination plan, timeline¹⁰

Data analysis. NIRS and ultrasonography data, expedition + 2 months.

Echocardiography, expedition + 4 months

Results discussion and article preparation. Article 1 (Effect of PEP on oxygenation and extravascular pulmonary fluid accumulation at high altitude), expedition + 5 months. Article 2 (Effect of hypoxia and PEP on cardiac function at high altitude), expedition + 6 months

Conference presentation and article submission. Expedition + 7-12 months

Research requirements, participants¹¹

Total time spent by one volunteer for this study: 1h45

Subjects will be continuously monitored during the evaluations with medical supervision. Risks expected for the participants are very few; discomfort while breathing with PEP could be improved at any time by removing the PEP device. Subjects will be requested not to perform intensive or prolonged physical exercise within 3h before the tests.

Research requirements, personnel¹²

4-5 researchers are required to run this experiment. Fully trained operators for echocardiography, ultrasonography and NIRS are needed. The entire group of experimenters should be able to evaluate two volunteers (shifted by 40 min) simultaneously, and therefore up to 10 subjects per day when needed.

Research requirements, equipment¹³

For PEP breathing: Hans Rudolph 3-way valve, Ambu PEEP[®] 0-10 cmH₂O (Ballerup, Danmark).

For cardiorespiratory monitoring: Heart rate and blood pressure measurements, pulse oxymetry, arterial (or capillary) blood sampling and analysis, breathing pattern and end-tidal CO₂ measurement (PM8000, Mindray, China; Power requirement: 100-240 VAC; Weight: 5 kg)

For thoracic ultrasonography: CX-50 (Phillips, Eindhoven, Netherlands; Power requirement: 100-240 VAC; Weight: 8 kg) with abdominal 5–2 MHz probe.

For echocardiography: Vivid Q (GE Healthcare, Horten, Norway; Power requirement: 100-240 VAC; Weight: 5 kg) with cardiac 3.5 MHz probe.
 For near infrared resonance spectroscopy: PortaLite (ARTINIS, The Netherlands; Power requirement: 100-240 VAC; Weight: 5 kg)
 For blood gas analysis: Gas analyzer (Radiometer, Copenhagen; Power requirement: 100-240 VAC; Weight: 10kg)
 For subject installation: Two mattresses in a tent.

Research requirements, consumables¹⁴

The only consumables are ECG electrodes, ultrasound gel, cleaning kits for masks and valves, materials for blood sampling and analysis.

Research requirements, logistics¹⁵

Equipment total weight: ~50 Kg
 Laboratory requirements: ~7-8 m², with electric supply, ambient temperature allowing subjects to remove upper clothes, water to clean masks and valves.

Research requirements, research cost¹⁶

All research devices are available within our laboratory or will be lent by companies (as in previous projects from our team), some spare parts (valves and PEP for instance) may be needed and will be budgeted for 10 000 €. This research cost will be met by industrial and local public institution sponsorship.

<p>[Print full name of principle investigator and collaborators here and all sign in next column]¹⁷</p>	<p>VERGES  RUPP  BOUZAT  WALTHER  ESTEVE </p>	<p>23-12-13</p>
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¹Title, full name, current post, department, institution, contact postal address, email address, telephone (including country and area code)

²Title, full name, department, institution, email address

³Max 20 words

⁴Project summary in simple English. Max 200 word

⁵Provide rationale for study

⁶Concise; specific and directional hypotheses

⁷Participants; research design; study schematic; procedures; statistical analyses; identification of main outcome measure; justification of sample size

⁸Graphs as likely to be presented in manuscript depicting theoretical relationships but correct units and physiologically plausible absolute values; explanatory text to justify relationships (based on previous literature)

⁹Target journal(s)

¹⁰Timeline from research proposal to submission of, manuscript to target journal (including conference presentations and 1st draft of introduction/methods/results/discussion sections)

¹¹Total time participants will spend on study; ¹²Risk to participants and how risks will be mitigated

¹²Staff required to run project successfully

¹³Make, model, where equipment will be sourced from, rough estimate of power requirements

¹⁴Plastics, paper, disposable accessories for equipment, etc

¹⁵Rough estimates of: sample transport (if required); equipment total weights; laboratory requirements (space, environmental conditions, services (water, electric, light, waste disposal)

¹⁶Direct expenditure related to project and explanation of how these costs will be met. Do not include expedition fees or logistics, or indirect salaries

¹⁷Principal Investigator and Collaborators must provide consent to submit proposal. This can be done with either physical or electronic signatures on the research proposal, or alternatively each researcher may email j.h.macdonald@bangor.ac.uk the following text: "I [INSERT NAME] approve the full research proposal entitled [INSERT TITLE]"

- Formatting
 - Please type information into table above and expand table as necessary
 - Min 12 point, min 1.5 line spacing, 2cm margins, times new roman, reference format as per Journal of Applied Physiology guidelines, include page numbers and principal investigator surname in a footer on every page; scientific proposal section should not exceed six pages of A4 plus references; research requirements should not exceed four pages of A4
- Submission
 - Email one pdf file to j.h.macdonald@bangor.ac.uk
 - Closing date: 24.12.13, 1200, Greenwich Mean Time
 - Please also ensure all researchers have read, completed and submitted form 3: researcher application form
 - Please also ensure the principle investigator has read, completed and submitted form 4: principal investigator contract.
 - Suggest at least four reviewers
 - Must have no known conflict of interest
 - Provide title, full name, position, department, institution, email address and phone number (including country and area code)
 - You will receive confirmation of submission within five working days
- Queries
 - Contact MEDEX Manaslu 2015 Research Lead
 - Jamie Macdonald PhD, Extremes Research Group, Bangor University
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